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IN VITRO EFFECTS OF METHYLPREDNISOLONE SODIUM SUCCINATE AND E. --ETC(U)
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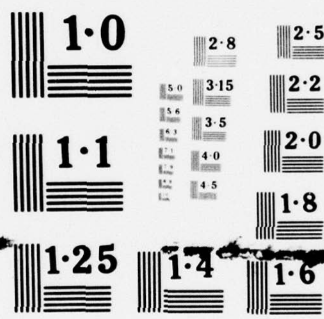

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Contract N00014-76-C-0229

Project No. NR 207-040

TECHNICAL REPORT NO. 122

IN VITRO EFFECTS OF METHYLPREDNISOLONE SODIUM SUCCINATE AND E. COLI ORGANISMS ON NEUTROPHILS IN BABOON BLOOD

L. B. Hinshaw, B. K. Beller, J. A. Majde,

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Prepared for Publication

in

Circulatory Shock

University of Oklahoma Health Sciences Center

~~Departments of Pathology & Physiology & Biophysics~~

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ABSTRACT

The corticosteroid, methylprednisolone sodium succinate (MP), has been observed to prevent hypoglycemia in experimental septic shock; however, detrimental actions of various corticosteroids on polymorphonuclear leukocyte function have been reported. The present study was designed to determine if MP depresses glucose metabolism of leukocytes or adversely affects neutrophil survival, or whether it modifies the mortality rate of live _E. coli_ in baboon blood _in vitro_. Results show that therapeutically effective concentrations (13 μg/ml blood) and high doses (130 μg/ml blood) of MP exert no detrimental influences on glucose utilization or survival of neutrophils in the absence or presence of _E. coli_ organisms in concentrations of 4.2×10^7 and 2.3×10^8 organisms/ml blood. _E. coli_ organisms, however, increase neutrophil mortality rate and glucose uptake of the blood. These findings support the view that MP does not adversely influence leukocyte metabolism and survival, nor does it modify the mortality rate of live _E. coli_.

KEY WORDS: septic shock, neutrophils, polymorphonuclear leukocytes, glucose uptake, baboon, _E. coli_ organisms, corticosteroids in shock, methylprednisolone, _in vitro_ blood study

INTRODUCTION

The incidence of septic shock has increased almost twenty-fold in the United States during the past twenty years (1), and its critical nature has been underscored in a recent review in which over 130,000 deaths per year are estimated to occur from a total number of 330,000 cases (1).

Experimental septic shock has been studied extensively in animals during the past several decades in order to understand the mechanism of septic shock in man. Pathophysiological manifestations in the nonhuman primate administered varying doses of live E. coli organisms include progressively developing systemic hypotension and hypoglycemia in all nonsurviving animals (2). Hypoglycemia has been reported in human septic shock in adults and children (3-5) and in the canine species administered lethal doses of E. coli organisms (6,7). Current findings strongly suggest that hypoglycemia results from the adverse effects of E. coli on hepatic gluconeogenesis (6) and from increased glucose utilization (8,9), including accelerated uptake by the blood in the canine species (10,11) and increased utilization in man (12,13).

Our recent studies were conducted on canine blood to which varying doses of live E. coli were administered in vitro. Results demonstrated consistent accelerated glucose disappearance at all doses of E. coli or endotoxin with significantly greater disappearance rates of glucose at higher dosages (10). Increased utilization of glucose was traced to the white blood cell population (10) with inference that the neutrophil was the cell responsible for the accelerated uptake.

Circulating phagocytic cells, particularly neutrophils, have been shown to exert a potent antibacterial defense in septic shock (14-16). Defects in neutrophil function are considered to have a significant negative impact on the ability to recover from bacterial infections, including septic shock (17,18).

The current consideration of the use of corticosteroids in the treatment of clinical and experimental septic shock is a confusing issue because of the conflict of findings in regard to therapeutic effects and a possible depressant action of the steroids on leukocyte function. Beneficial actions of glucocorticoids have been reported for the treatment of septic shock in animals (19) and man (20). Several studies, however, have described adverse effects of steroids on polymorphonuclear leukocyte function, including inhibition of chemotaxis (21,22), depression of mobilization (23) and diminished bactericidal capability (24-28). Other reports have shown that certain corticosteroids exert no adverse effects on the bactericidal activities of leukocytes (27,29), including engulfment and killing of enteric bacteria (30), or may enhance their function (31).

The purpose of the present study was to answer the questions, "Does the steroid methylprednisolone sodium succinate (MP) depress glucose metabolism of leukocytes?"; "Does the addition of MP adversely affect neutrophil survival?"; and "Does MP modify the survival rate of live *E. coli* added to blood *in vitro*?". Results from these experiments provide evidence for the absence of adverse effects of MP on neutrophil metabolism and survival and indicate that its presence neither accelerates nor depresses the survival rate of live *E. coli* organisms.

METHODS

In vitro studies were carried out on the blood from six adult baboons of random sex weighing between 12.6 and 16.2 kg. Each animal was fasted overnight, restrained by means of a squeeze cage device the next morning and administered ketamine hydrochloride (Ketaset; Bristol-Myers Co., Syracuse, N.Y.), 5 mg/kg, intramuscularly. Within 5-10 minutes the baboon was gently lifted

from the cage and blood was drawn from a femoral vein with a needle and plastic syringe wet with heparin (1000 U/ml). The blood was immediately divided into 5 ml volumes in six separate plastic tubes each containing 0.05 ml of heparin (20,000 U/ml), capped, gently mixed and placed on ice. Appropriate volumes of saline or saline plus low or high concentrations of methylprednisolone sodium succinate were then added to three of the tubes of blood, while the remaining three tubes received live E. coli organisms alone or together with low or high doses of the steroid. The six tubes of blood from the same baboon were then simultaneously placed in a water bath at 37°-38°C and incubated. In vitro blood glucose concentrations were determined hourly with a Beckman glucose analyzer (Beckman Instruments; Fullerton,Ca.) possessing an accuracy of ±3 mg%, and each experiment was terminated when blood glucose concentrations fell to an average concentration of 14 mg% (SE, ±1). Initial and final white blood cell (WBC) counts were measured with an automatic particle counter (Coulter Z_F; Hialeah, Fla.), and the WBC differentials were estimated by microscopic examination of blood smears stained with Wright's stain.

Preparation of the live organisms for use in these studies was as follows: E. coli, Type B, isolated from a stool specimen at Childrens Memorial Hospital, Oklahoma City, Okla., was maintained in the lyophilized state at 4°C after growth on tryptic soy agar (TSA). The E. coli was reconstituted as needed with 2-3 ml tryptic soy broth and incubated at 37°C for 4-6 hours. Fresh TSA slants were inoculated from the broth suspension using a sterile cotton swab and incubated at 37°C for approximately 18 hours. The cells were then washed from the slants with 2-3 ml of physiological sterile saline (PSS). The washing was centrifuged, the supernatant was discarded and the cells were resuspended in PSS. The cell suspension was then adjusted with PSS to a predetermined

density monitored by reading percentage of transmittance on a Coleman spectrophotometer. _E. coli_ viability counts were done at the beginning and end of all _in vitro_ experiments, using standard serial dilution and pour-plate techniques.

These experiments were designed to evaluate the separate effects of low and high doses of live _E. coli_ organisms and the corticosteroid, methylprednisolone sodium succinate, on glucose uptake and survival of polymorphonuclear leukocytes, specifically neutrophils, in baboon blood _in vitro_. Accelerated uptake of glucose by the blood in response to _E. coli_ addition was ascribed to increased metabolic activity of the white blood cells in earlier reports (10,32). _E. coli_ concentrations were calculated from data on intact baboon experiments conducted in this laboratory (2). The LD_{100}, $1x10^{10}$ organisms/kg, determined in the earlier _in vivo_ studies, was calculated for use in the present _in vitro_ studies by assuming an _in vivo_ blood volume of 75 ml/kg and instantaneous mixing of organisms _in vivo_, and the _in vitro_ "high concentration" thus selected was $1.3x10^{8}$ organisms/ml blood. The "low concentration", 1/5 LD_{100}, was utilized in order not to overwhelm the phagocytic capacity of neutrophils (70(±12) organisms per neutrophil--LD_{100}--vs. 19(±3) organisms per neutrophil--1/5 LD_{100}). Methylprednisolone sodium succinate (Solu-Medrol; The Upjohn Company, Kalamazoo, Mich.) was added to blood samples with and without _E. coli_ organisms in concentrations equaling the _in vivo_ half-life concentration in dogs, 13 μg/ml serum (unpublished data from The Upjohn Company), and ten times this dosage, 130 μg/ml, the latter chosen to determine if high levels of the steroid would be detrimental to the neutrophil. Six samples of blood from the same baboon were simultaneously incubated during a 3-6 hour period, three samples containing saline alone or with low or high doses of steroid, and three receiving _E. coli_ alone or _E. coli_ together with low or high doses of steroid. Blood from the same baboon was alternately used in separate experiments with

high or low doses of *E. coli* separately added to the blood. Results from all experiments were analyzed using the *t* test for paired or unpaired data.

RESULTS

Figures 1A and 1B depict *in vitro* effects of varying methylprednisolone sodium succinate (MP) and live *E. coli* organism concentrations on mean cumulative glucose uptake in the blood from six baboons. Results from Figure 1A indicate that neither low nor high concentrations of MP depress glucose uptake of the blood in the absence of *E. coli* organisms (lower curve); however, during the first two hours glucose utilization increases in contrast to the saline controls ($p<0.005$). The presence of live organisms (LD_{100}; upper curves) results in a markedly elevated glucose uptake during all time periods ($p<0.05$). High concentrations of MP increase cumulative glucose uptake by the end of the second and third hours ($p<0.05$). Figure 1B illustrates the *in vitro* effects of MP and 1/5 LD_{100} live *E. coli* organisms on mean cumulative glucose utilization in the blood from six baboons. Results in the lower three sets of curves in which MP alone was added to blood are indistinguishable from those in Figure 1A (lower curves), the studies having been carried out on the original six baboons, but on different days. In distinction from the results shown in Figure 1A, the addition of a lower dose of *E. coli* elicits a lesser, though significant, effect on accelerating glucose utilization ($p<0.05$) with the exception of the first hour. Also, as was the case in Figure 1A, the presence of MP in higher concentrations results in increased glucose utilization in the presence of live organisms ($p<0.05$). Findings from these data show that neither low nor high concentrations of methylprednisolone depress the cumulative uptake of glucose by the blood during a 5-hour period in the absence or presence of less than lethal to LD_{100} concentrations of live *E. coli*

organisms. The addition of organisms, however, elicits notable increases in glucose utilization, quantities approximately doubling in the presence of high concentrations (2.3×10^8 organisms/ml).

The question as to possible effects of MP on neutrophil survival was addressed by data shown in Tables IA and IB. Table IA presents findings pertaining to the *in vitro* effects of MP and LD_{100} live *E. coli* organisms on the blood neutrophil concentration in six baboons. Results show that low and high concentrations of MP do not alter the survival rate of mature and immature neutrophils during a mean *in vitro* exposure time of 2.6 hours. Addition of *E. coli* organisms in an LD_{100} concentration of 2.3×10^8 organisms/ml blood markedly decreases the numbers of mature neutrophils ($p<0.02$), and the numbers of degenerated neutrophils are significantly elevated within 2.6 hours ($p<0.05$). The addition of low and high concentrations of MP to blood containing lethal concentrations of *E. coli* did not alter the numbers of mature, immature and degenerated neutrophils in comparison to blood containing *E. coli* alone ($p>0.05$). Table IB provides data for the *in vitro* effects of MP and 1/5 LD_{100} live *E. coli* organisms on neutrophil mortality in baboon blood. Results show that the addition of low and high concentrations of MP does not alter the survival rate of mature and immature neutrophils during a mean exposure time of 4.3 hours. The duration of experiments was based on the time required for blood glucose to fall below 20 mg%, and times were consistently observed to be directly related to the concentration of *E. coli* (see Table IA for comparison). Addition of live organisms at a 1/5 LD_{100} concentration (4.2×10^7/ml blood) decreased the numbers of mature neutrophils ($p<0.01$) and increased the quantities of degenerated neutrophils ($p<0.02$). Addition of low and high concentrations of MP to blood in the presence of *E. coli* did not change the numbers of mature, immature and degenerated neutrophils in comparison to blood containing *E. coli*

alone ($p>0.05$). These findings indicate that although varying doses of E. coli markedly depress the concentrations of mature neutrophils, incubation with either low or high concentrations of methylprednisolone has no effect on the survival rate of neutrophils. The steroid added to blood in the absence of E. coli does not accelerate the loss of mature and immature neutrophils.

Table II illustrates the effect of MP on E. coli mortality in the blood from six baboons observed in vitro. Results show that high or low concentrations of MP added to blood in the presence of LD_{100} and 1/5 LD_{100} quantities of live E. coli do not influence the mortality rate of E. coli during incubation times of 2.6 and 4.3 hours, respectively. Mean concentrations of organisms decreased between 40 and 50% during a 2.6-hour period of incubation with LD_{100} concentrations of E. coli with and without MP and decreased between 16 and 46% with lower concentrations of live organisms during a 4-hour period. These findings indicate that neither low nor high blood concentrations of methylprednisolone exert positive or negative influences on the E. coli mortality rate in vitro.

It was thought to be of interest to determine the effects of MP and E. coli organisms on lymphocyte mortality. Initial concentrations of lymphocytes were compared to final concentrations in each of 12 types of experiments (N=6 in each of the 12 experiments). Table III demonstrates that the numbers of lymphocytes per mm^3 of blood remain relatively constant in all conditions of the experiments during mean incubation periods of 2.6 and 4.3 hours. The only change was a significant increase in mean lymphocyte concentration in the study utilizing the combination of high doses of E. coli and low concentrations of MP ($p<0.05$), which presumably was due to limitations of accuracy in the counting method.

DISCUSSION

The increasing incidence of septic shock (1) must reflect in part the lack of understanding of its underlying mechanisms. Recent work has emphasized metabolic defects in both its experimental and clinical forms, and in particular

the role of glucose has been emphasized (33). Hypoglycemia has been reported in dogs and nonhuman primates administered live E. coli organisms (2,6,7) and in children and adults subjected to septic shock (3-5). The hypoglycemia is not necessarily a terminal event but may occur during the early course of shock in nonsurviving animals (2,7). Available data suggest that the primary cause of hypoglycemia is failure of hepatic gluconeogenesis (6), and although increased glucose utilization is suspected in clinical septic shock (12,13), published reports from canine studies have documented a significant disappearance of glucose in the blood following live E. coli administration (10,11). In the latter studies (10,11), the white blood cell population was responsible for the accelerated uptake of glucose, and observations implicated the neutrophil as the primary cell accountable. The neutrophil has been shown to exert a potent antibacterial defense in septic shock (14-16,34,35), and defects in its function have been reported to adversely influence the course of bacterial infections and septic shock (17,18). It appears that the circulating phagocyte provides an important defense for the host (14,36,37) but exacts a high metabolic cost in the performance of its role (10,11,38).

Corticosteroids have been studied extensively as a mode of treatment in experimental or clinical septic shock, and although findings are controversial, recent reports with more refined criteria for administration of the agent and its evaluation have shown it to exert a beneficial effect on survival (19,20). However, the actions of steroids on neutrophil function are uncertain since varying adverse effects, including defective mobilization, inhibition of chemotaxis and depression of bactericidal capacity, have been reported (21,22,24-28).

It was thought to be important to characterize the effects of a prominently utilized corticosteroid, methylprednisolone sodium succinate, on neutrophil function and survival and on the mortality rate of live E. coli organisms added

to baboon blood in an in vitro system. Experiments were designed to answer the following questions, "Does methylprednisolone (MP) depress glucose metabolism of leukocytes and survival of neutrophils, and is it able to modify the survival rate of live E. coli?" Differing concentrations of steroid and organisms were employed in order to include most experimental or clinical eventualities. Blood from the same baboons was used in all procedural maneuvers in order to reduce experimental variables to a minimum. The first question to be answered by the present study was, "Does MP depress glucose metabolism of leukocytes?" Findings show that the presence of both low and high concentrations of MP does not depress the uptake of glucose in the blood during a 3-5 hour period in the absence or presence of lethal to less than lethal ($<LD_{100}$) concentrations of live E. coli organisms. The presence of MP with or without E. coli organisms increases cumulative uptake of glucose by the blood during significant portions of the incubation period. The second question addressed in the present study was, "Does the addition of MP adversely affect neutrophil survival?" Findings indicate that incubation of blood in the absence of E. coli organisms with low or high concentrations of MP does not affect the survival rate of mature and immature neutrophils. Adding low or high doses of live E. coli to blood markedly decreases the concentrations of mature neutrophils, but adding MP has no effect on the survival rate of neutrophils. The final question to be answered was, "Does MP modify the survival rate of live E. coli added to blood in vitro?" Findings from the present study showed that low or high concentrations of MP exert neither positive nor negative influences on E. coli mortality. Although E. coli concentrations decreased significantly during incubation with blood, MP had no influence on mortality rates of the organisms during a 3-5 hour period of observation.

Results from the present study suggest that the corticosteroid, methylprednisolone sodium succinate, employed in therapeutic concentrations or greatly exceeding these levels, exerts no demonstrable detrimental direct effects on neutrophil glucose metabolism or neutrophil survival. The introduction of live _E. coli_ into baboon blood results in a marked increase in glucose consumption, confirming earlier studies using _E. coli_ or endotoxin alone in canine blood (10, 11), and the presence of organisms greatly elevates the mortality rate of neutrophils, which is unaltered by the presence of the steroid in low and high concentrations. In addition to observations on neutrophils, concentrations of lymphocytes remained relatively constant in all experiments. Since the data presented in the present study are confined to _in vitro_ conditions and do not evaluate possible indirect effects of this steroid on blood leukocytes, their application to the _in vivo_ state must await further investigation.

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TABLE IA. *In Vitro* Effects of Methylprednisolone Sodium Succinate (MP) and LD_{100} *E. coli* Organisms* on Neutrophil Concentration in Baboon Blood (Mean±SE; N=6, each group)

Experiment	Neutrophils: Mature	Immature	Degenerated	Total†	Total WBC (#/mm³)
Initial Concentrations of Neutrophils (#/mm³; zero time; Mean±SE)					
All experiments	3738(896)	225(100)	0(0)	3964(904)	7504(899)
Final Concentrations of Neutrophils (#/mm³; +2.6±0.2 hours; Mean±SE)					
Blood without *E. coli*					
a) Saline only	3960(776)	160(64)	0(0)	4394(802)	7817(747)
b) MP (13 µg/ml)	3838(690)	151(68)	41(41)	4030(644)	7933(744)
c) MP (130 µg/ml)	3830(597)	135(63)	52(52)	4034(666)	7817(639)
(a) compared to (b)	NS	NS	NS	NS	p<0.05
(a) compared to (c)	NS	NS	NS	NS	NS
Blood with *E. coli*					
d) Saline + *E. coli*	1043(208)§	104(38)	1731(650)§	2878(631)§	7233(494)
e) MP (13 µg/ml) + *E. coli*	1295(217)§	117(45)	1364(542)	2776(482)	7150(557)
f) MP (130 µg/ml) + *E. coli*	1333(391)§	118(83)	1829(368)§	3280(497)	7400(604)
(d) compared to (e)	NS	NS	NS	NS	NS
(d) compared to (f)	NS	NS	NS	NS	NS
Statistical Analysis of Effect of *E. coli* on Concentration of Neutrophils					
(a) compared to (d)	p<0.02	NS	p<0.05	p<0.05	NS
(b) compared to (e)	p<0.025	NS	NS	p<0.005	p<0.05
(c) compared to (f)	p<0.005	NS	p<0.005	p<0.05	p<0.05

*2.3 X 10^8 *E. coli*/ml blood (LD_{100})

†Total = mature + immature + degenerated neutrophils

§$p \leq 0.05$, initial compared to final concentrations

TABLE IB. *In Vitro* Effects of Methylprednisolone Sodium Succinate (MP) and One-Fifth (1/5) LD_{100} *E. coli* Organisms* on Neutrophil Mortality in Baboon Blood (Mean±SE; N=6, each group)

Experiment	Neutrophils: Mature	Immature	Degenerated	Total†	Total WBC (#/mm³)
Initial Concentrations of Neutrophils (#/mm³; zero time; Mean±SE)					
All experiments	2329(401)	86(44)	20(20)	2434(402)	5776(330)
Final Concentrations of Neutrophils (#/mm³; +4.3±0.3 hours; Mean±SE)					
Blood without *E. coli*					
a) Saline only	2530(467)	54(27)	39(28)	2623(470)	6067(367)§
b) MP (13 μg/ml)	2550(394)	72(18)	31(22)	2653(400)	6233(352)§
c) MP (130 μg/ml)	2274(395)	58(31)	116(66)	2448(355)	5983(309)§
(a) compared to (b)	NS	NS	NS	NS	NS
(a) compared to (c)	NS	NS	NS	NS	NS
Blood with *E. coli*					
d) Saline + *E. coli*	1436(426)§	33(24)	821(210)§	2288(495)	5633(409)
e) MP (13 μg/ml) + *E. coli*	1690(220)	141(73)	479(209)	2309(333)	5750(331)
f) MP (130 μg/ml) + *E. coli*	1497(310)§	59(27)	688(324)	2244(592)	5817(399)
(d) compared to (e)	NS	NS	NS	NS	NS
(d) compared to (f)	NS	NS	NS	NS	NS
Statistical Analysis of Effect of *E. coli* on Concentration of Neutrophils					
(a) compared to (d)	p<0.01	NS	p<0.02	NS	NS
(b) compared to (e)	p<0.02	NS	NS	NS	p<0.001
(c) compared to (f)	p<0.025	NS	NS	NS	NS

*4.2 X 10^7 *E. coli*/ml blood (1/5 LD_{100})

†Total = mature + immature + degenerated neutrophils

§$p \leq 0.05$, initial compared to final concentrations (limitation of method, ±600 cells/mm³; Coulter Diagnostics, Inc., Hialeah, Florida)

TABLE II. Effect of Methylprednisolone Sodium Succinate on E. coli Mortality in Baboon Blood In Vitro

(Mean ±SE; N=6, each group)

High concentration, E. coli				Low concentration, E. coli			
Initial mean concentration (#E. coli/ml)	Final mean concentration (#E. coli/ml)	% change	Time of incubation (hrs)	Initial mean concentration (#E. coli/ml)	Final mean concentration (#E. coli/ml)	% change	Time of incubation (hrs)
Control (no methylprednisolone)							
2.3×10^8 [a]	1.3×10^8 [b]	-42.3 [c]	2.6	4.2×10^7 [a]	4.0×10^7 [b]	-16.4 [d]	4.3
(1.0×10^7)	(9.0×10^7)	(37.5)	(0.2)	(0.5×10^7)	(2.7×10^7)	(53.8)	(0.3)
Methylprednisolone concentration = 13 g/ml							
2.3×10^8 [a]	1.2×10^8 [b]	-50.3 [c]	2.6	4.2×10^7 [a]	2.6×10^7 [b]	-46.4 [d]	4.3
(1.0×10^7)	(7.4×10^7)	(30.8)	(0.2)	(0.5×10^7)	(1.8×10^7)	(35.2)	(0.3)
Methylprednisolone concentration = 130 g/ml							
2.3×10^8 [a]	1.4×10^8 [b]	-39.6 [c]	2.6	4.2×10^7 [a]	2.8×10^7 [b]	-43.8 [d]	4.3
(1.0×10^7)	(7.1×10^7)	(29.6)	(0.2)	(0.5×10^7)	(2.0×10^7)	(37.6)	(0.3)

[a] Significant difference between initial values of low and high E. coli concentrations ($p<0.001$).

[b] No significant difference between final values of low and high E. coli concentrations ($p>0.05$).

[c] No significant difference in percent changes between control and methylprednisolone-treated groups.

[d] No significant difference in percent changes between control and methylprednisolone-treated groups.

TABLE III. *In Vitro* Effects of Methylprednisolone Sodium Succinate (MP) and *E. coli* Organisms on Lymphocyte Concentration in Baboon Blood (Mean±SE; N=6, each group)

High Dose, *E. coli*			Low Dose, *E. coli*		
Mean initial concentration of lymphocytes, $3410 \pm 467/mm^3$			Mean initial concentration of lymphocytes, $3459 \pm 634/mm^3$		
Experiment	Final concentration of lymphocytes (termination of experiment; $\#/mm^3 \pm SE$)	Significance*	Experiment	Final concentration of lymphocytes (termination of experiment; $\#/mm^3 \pm SE$)	Significance
Saline (no *E. coli*)	3248(288)	NS	Saline (no *E. coli*)	3138(288)	NS
MP (13 μg/ml) (no *E. coli*)	3445(335)	NS	MP (13 μg/ml) (no *E. coli*)	3373(237)	NS
MP (130 μg/ml) (no *E. coli*)	3469(524)	NS	MP (130 μg/ml) (no *E. coli*)	3246(279)	NS
E. coli (2.3×10^8/ml) + saline	3930(426)	NS	*E. coli* (4.2×10^7/ml) + saline	3098(228)	NS
E. coli (2.3×10^8/ml) + MP (13 μg/ml)	3851(439)	$p<0.05$†	*E. coli* (4.2×10^7/ml) + MP (13 μg/ml)	3043(196)	NS
E. coli (2.3x10 /ml) + MP (130 μg/ml)	3624(319)	NS	*E. coli* (4.2×10^7/ml) + MP (130 μg/ml)	3264(357)	NS

*Statistical significance between initial and final concentrations in each of the 12 sets of experiments. (Mean times of incubation: high dose *E. coli* group = 2.6±0.2 hr; low dose *E. coli* group = 4.3±0.3 hr).

†Limitation of method, ±600 cells/mm^3 (see Table 1B).

LEGENDS FOR FIGURES

Figure 1A. *In vitro* effects of methylprednisolone sodium succinate (MP) and LD_{100} *E. coli* organisms on glucose utilization in baboon blood (six experiments, N=6 each experiment).

* per ml blood

** per ml blood - LD_{100}

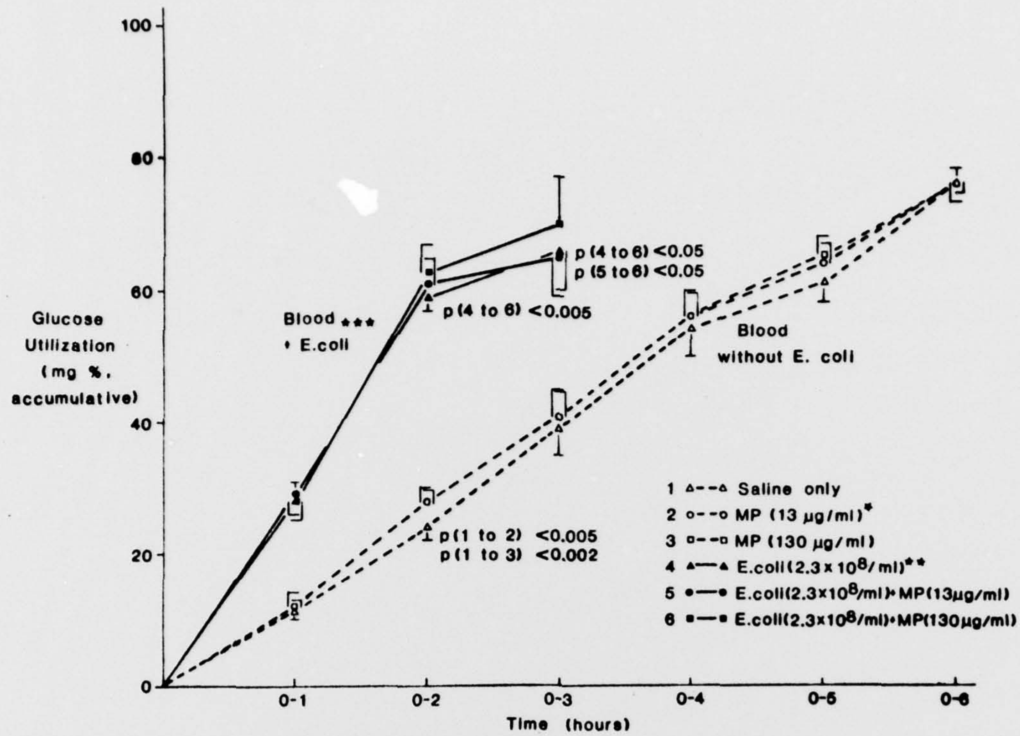
*** addition of *E. coli* results in increased glucose utilization, hours 1,2,3 ($p \le 0.05$)

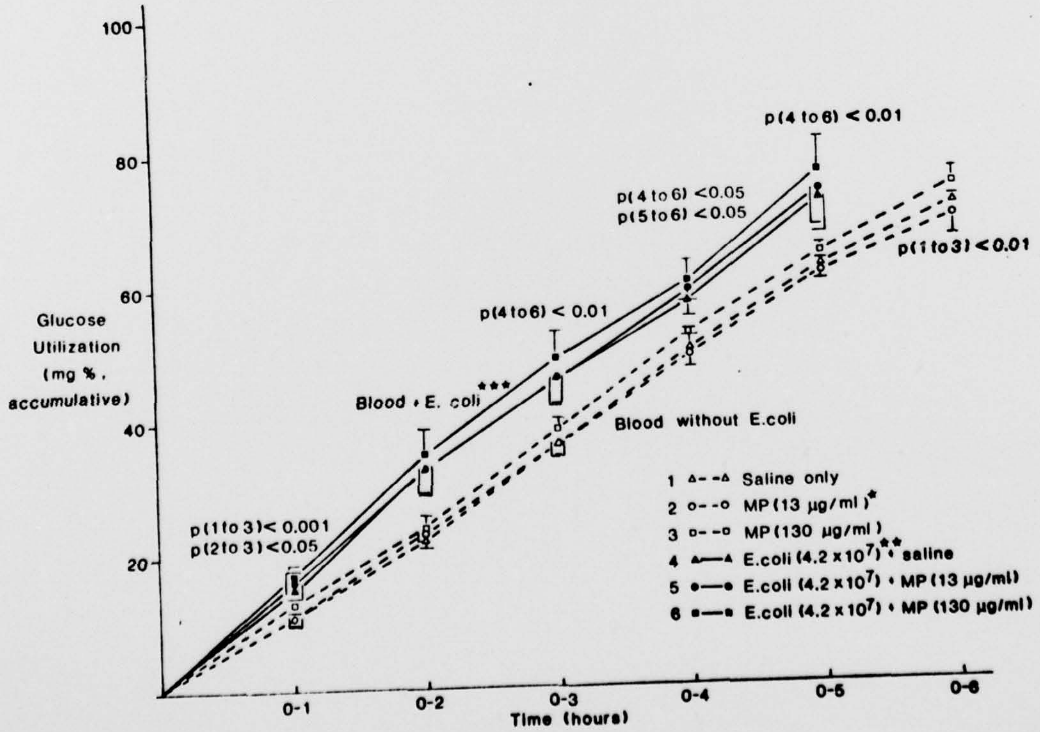
Figure 1B. *In vitro* effects of methylprednisolone sodium succinate (MP) and 1/5 LD_{100} *E. coli* organisms on glucose utilization in baboon blood (six experiments, N=6 each experiment).

* per ml blood

** per ml blood - $<LD_{100}$

*** addition of E. coli results in increased glucose utilization, hours 2,3,4 ($p \le 0.05$)


Glucose Utilization (mg %, accumulative)
Time (hours)
Blood *** + E.coli
Blood without E. coli
p (4 to 6) <0.05
p (5 to 6) <0.05
p (4 to 6) <0.005
p (1 to 2) <0.005
p (1 to 3) <0.002
1 Saline only
2 MP (13 μg/ml)*
3 MP (130 μg/ml)
4 E.coli (2.3 x 10^8/ml)**
5 E.coli (2.3 x 10^8/ml) + MP (13 μg/ml)
6 E.coli (2.3 x 10^8/ml) + MP (130 μg/ml)


Glucose Utilization (mg %, accumulative)
Time (hours)
p (4 to 6) < 0.01
p (4 to 6) <0.05
p (5 to 6) <0.05
p (1 to 3) < 0.01
p (4 to 6) < 0.01
Blood + E. coli ***
Blood without E.coli
p (1 to 3) < 0.001
p (2 to 3) < 0.05
1 Saline only
2 MP (13 μg/ml)*
3 MP (130 μg/ml)
4 E.coli (4.2 x 10^7)** + saline
5 E.coli (4.2 x 10^7) + MP (13 μg/ml)
6 E.coli (4.2 x 10^7) + MP (130 μg/ml)

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13. ABSTRACT

The corticosteroid, methylprednisolone sodium succinate (MP), has been observed to prevent hypoglycemia in experimental septic shock; however, detrimental actions of various corticosteroids on polymorphonuclear leukocyte function have been reported. The present study was designed to determine if MP depresses glucose metabolism of leukocytes or adversely affects neutrophil survival, or whether it modifies the mortality rate of live E. coli in baboon blood in vitro. Results show that therapeutically effective concentrations (13 μg/ml blood) and high doses (130 μg/ml blood) of MP exert no detrimental influences on glucose utilization or survival of neutrophils in the absence or presence of E. coli organisms in concentrations of 4.2×10^7 and 2.3×10^8 organisms/ml blood. E. coli organisms, however, increase neutrophil mortality rate and glucose uptake of the blood. These findings support the view that MP does not adversely influence leukocyte metabolism and survival, nor does it modify the mortality rate of live E. coli.

10 to the 8th power

10 to the 7th power

microgram

microgram

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